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Chapter 5

PHYSICAL PROPERTIES OF POLYMER-GRAFTED BILAYERS

Kalina Hristova and David Needham

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I. INTRODUCTION

The behavior of solvated polymers that are adsorbed or grafted at the interface between biofluids and biomaterials is of interest to polymer physicists and bioengineers alike because of the unique repulsive properties that these polymers possess. For example, a strategy based on the conformation of water-soluble polymers grafted to lipid bilayers is leading to a more effective intravenous liposome drug delivery system.[1-7] As described elsewhere in this volume, drugs and conventional liposomes are rapidly cleared from the bloodstream by the reticuloendothelial system and other nonspecific mechanisms.[8] However, this clearance of liposomes can be significantly decreased by incorporating polyethylene glycol-linked lipids (molecular weights 2000 and 5000 Da). This leads to a substantial increase in the blood circulation time of these so-called "Stealth" liposomes.* It is believed that the mechanism of stabilization is a physical one:[1,2,4,5] the polymer creates a steric barrier to enhance the repulsive

* "Stealth" is a trademark of Liposome Technology, Inc.

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properties of the liposomal surface, stabilizing the lipid bilayer against close approach of other macromolecules and cells. The application has driven recent interest in the basic physical properties of these interfacial structures.

For *in vivo* applications, the polymer-grafted liposomes are formed by spontaneous rehydration of a polymer-lipid/lipid mixture (for instance, distearoyl phosphatidyl-ethanolamine-polyethyleneglycol/distearoyl phosphatidylcholine) in which the concentrations of polymer-lipids in the lipid mixture are less than 15 *M%*.[9,10] To characterize these so-formed polymer-grafted bilayers we use several different experimental methods that give information about the physical properties of the polymer-lipid/lipid system (i.e., X-ray diffraction, micropipette manipulations, nuclear magnetic resonance (NMR), calorimetry, electron and optical microscopy.[4,9,10] Of these the most powerful so far has been X-ray diffraction (see Chapter 7, McIntosh et al.). As discussed in Reference 4 and Chapter 7, the X-ray diffraction method gives information about: (1) the extension length of the polymer away from the lipid surface, (2) the repulsive pressure with which the grafted polymer opposes mutual surface compression, and (3) structural information about the lipid phase and indication of phase separation if it occurs.

The micropipette technique allows us to measure: (1) bilayer elastic constants, (2) mechanical stability of the bilayer, and (3) interbilayer adhesion energies. Together with these two techniques, the other spectroscopic and microscopic methods allow us to confirm the phase behavior of the lipid/polymer-lipid/water system.

With this experimental data we are now in the process of testing theoretical models (existing and new) that describe the combined influence of the two components: the lipid bilayer and the polymer-grafted layer.

There is already a significant literature regarding both components of the system as separate entities. The physical properties of lipid bilayers have been studied and different experimental techniques have been developed to test existing theories.[11-22] Also, there have been recent developments in polymer physics, concerning the properties of polymers adsorbed to,[23-26] depleted from,[24,25,27] or grafted on solid surfaces.[28-34] Some of these theories have been tested and partially confirmed.[26,35-40] The repulsive features have mostly concerned polymers adsorbed or grafted to solid surfaces.

However, to fully understand the influence of polymer-grafted lipids on the physical properties of lipid bilayers, we have begun to develop a theory that coherently merges membrane and polymer physics. This theory should prove useful in the further understanding of the properties of biosurfaces and the design of systems that will interact with them.

Here we describe theoretical predictions for the influence of the polymer-bearing lipids on the interactive and structural properties of lipid bilayers. For two bilayers in close proximity, we model the interbilayer steric interactive forces for different polymer-lipid concentrations. These predictions are relevant for polymer-lipid concentrations in the range 0 to 10 *M%*, when the bilayer structure is the stable phase. At the other concentration extreme of the phase diagram, 100% polymer-lipid in water forms micelles.[9] This implies that, at intermediate compositions, the lipid/PEG-lipid/water system has a complex phase behavior and so the conditions under which bilayers (i.e., liposomes) are formed have to be determined. Special attention therefore has to be paid to the self-assembling properties of the polymer-lipid/lipid system.

II. BACKGROUND ON PHYSICAL PROPERTIES OF LIPID BILAYERS THAT ARE IMPORTANT TO LIPOSOME DESIGN

Amphiphilic molecules spontaneously self-assemble in aqueous media into aggregates such as bilayers and micelles. This occurs when the concentration exceeds the critical micelle or critical bilayer concentration, respectively (CMC or CBC) (for instance, for distearoyl

phosphatidylcholine, DSPC, the CBC is on the order of 10^{-10} *M*). Depending on the structure of the molecules and their concentration these aggregates can have different shapes (spherical, cylindrical, flat) and sizes. The common feature of the aggregates is that the polar (or hydrophilic) parts of the molecules are exposed to water and the apolar (or hydrophobic) parts are packed together and hidden inside the aggregate. The driving force for this self-assembly is an entropy-driven effect, called the hydrophobic effect.[41] It stems from the fact that immersing a hydrophobic object into a highly polar medium (such as water) leads to a different arrangement of the water molecules around the object than that of pure water and thus to a decrease in the entropy of the system. Thus, an aggregate exists mainly due to the fact that it is excluded from the solvent, since its molecules are not strongly bound — molecule-molecule interactions are of the weak van der Waals type. This is quantitatively manifested in the low elastic coefficients: (1) bending rigidity k_c and (2) area expansion modulus K_A in relation to other, more traditional engineering materials.[42]

For liquid bilayers k_c is of the order of 10^{-19} J. Typical values for the area compressibility modulus of fluid bilayers are $K_A \sim 0.2$ Nm^{-1} or 200 dyn/cm.[12] If we convert this surface compressibility into an equivalent bulk modulus by dividing by the membrane thickness ~5 nm, we get a compressibility ~10^7 Nm^{-2}, which is somewhere between that of an ordinary liquid and a gas. So we can view the membrane as a two-dimensional liquid that can be about 100 times more compressible than its embedding fluid.

Changing the cholesterol content (up to 50%) increases bilayer cohesion and dramatically changes bilayer physical properties (for instance, the incorporation of 50 *M*% cholesterol in SOPC membranes increases K_A by an order of magnitude and increases their strength by about six times).[15] Thus, varying the amount of cholesterol in the bilayer provides us with a simple means to vary the mechanical parameters (elastic constants and failure) of bilayers.

At a certain applied lateral isotropic tension τ_s (the tensile strength) a sudden and unavoidable membrane rupture occurs. At the point of rupture the membrane is characterized by its maximal area change. Expansions bigger than this one cannot be achieved. The tensile strength τ_s of the bilayer and the maximum area change have been measured in micropipette experiments[15,22] as a function of cholesterol content. Thus, tensile strengths for purely lipid bilayers are ~1 to 6 dyn/cm. Addition of cholesterol, especially to saturated lipids, increases τ_s to ~50 dyn/cm.[42] Critical areal strains are 1 to 5% and reflect complete lack of "ductility" in these two-dimensional materials. For gel-phase bilayers the mechanism of rupture will depend on the presence of defects and is not so well understood.

Bilayers can also undergo substantial changes in physical state with temperature. Above a certain transition temperature the bilayer exists as a two-dimensional liquid. Below this temperature the bilayer is a two-dimensional solid and the lipid molecules are tightly packed in a crystal lattice. Compared to their liquid crystalline state, K_A for gel-phase bilayers is about five times higher.[22] This gel-liquid crystalline transition temperature varies with the chemical composition and hydration level of the membrane and so is another way of controlling the physical state of a liposome capsule.

The rehydration of a mixture of polymer-lipids and lipids will lead to their spontaneous assembly into bilayer or micellar aggregates. If the hydrocarbon tails of the lipids and polymer-lipids are identical, one could expect the two types of lipids to mix well. In these aggregates, the hydrocarbon chains of the polymer-lipids will be packed together and the polar heads with their long hydrophilic polymer chains will be exposed to water. Since polyethylene glycol (PEG) does not adsorb on lipid surfaces,[26] it will remain attached to the bilayer at only one point (i.e., grafted, but not adsorbed to the bilayer). The chain will extend into the aqueous medium, thus maximizing its interaction with the solvent.

If the polymer is grafted on a *solid* surface the lateral repulsion between the polymer chains is unlikely to have any effect on the structure of the solid since the building units of solids are held together by strong chemical forces. In contrast, when the "substrate" is a lipid bilayer

polymer chain repulsion may create membrane curvatures, area expansion (increased area per molecule), or even changes of phase (bilayer to micelle) due to the low elastic coefficients and weak bonds between the lipid molecules. To understand the influence of the polymer on the bilayer we have to therefore also characterize the polymer system itself.

The behavior of polymer chains grafted on solid surfaces was first studied by de Gennes.[28] According to him, two concentration regimes can be identified for the polymer chains at the surface, and the physical characteristics of the polymer system are different in these two regimes. When the grafting density is low, the chains form separate "mushrooms", each with a size $R_F \cong aN^{3/5}$, where N is the degree of polymerization and a is the monomer size.* Such a situation is shown for a polymer-lipid bilayer system in Figure 1a. When the grafting level is high, the chains overlap laterally to form a continuous "brush" and the polymer system can now be considered a semidilute solution. The brush regime is shown in Figure 1b. Brushes have been described with scaling and mean-field theories. In the scaling scheme[28-30,43,44] it is supposed that the monomer concentration is constant throughout the polymer layer. In the mean-field scheme Milner et al.[31-34] include the possibility that a chain may not be uniformly stretched so that the monomer concentration varies throughout the polymer layer. They show that the self-similar concentration profile is parabolic and the brush is "softer" upon compression than predicted by scaling arguments.

We can apply these theoretical studies of polymers grafted on solid surfaces to the bilayer system. However, due to the characteristics of the bilayer as a self-assembling system a new phenomenon occurs: the polymer perturbs the surface it is grafted to, which in turn affects the characteristics of the polymer.

We will now consider the consequences of this mutual interaction between the grafted polymer and the lipid bilayer. These considerations will include the nature of the steric barrier that the polymer layer provides when two such bilayers interact, and the phase behavior of the polymer-lipid/lipid/water system. The influence of the polymer on the mechanical properties of the bilayer is considered in detail elsewhere.[45]

III. POLYMER STERIC INTERACTIONS: POLYMER EXTENSION LENGTH AND POLYMER COMPRESSIBILITY

When two polymer-grafted bilayers are opposed and made to approach each other under applied compressive osmotic forces (as in X-ray diffraction experiments,[4,9] the nature of the pressure vs. distance relation that characterizes polymer compressibility is dependent on polymer surface concentration. As discussed, two regimes can be identified: dilute mushrooms and dense brushes. Expressions for polymer extensions away from the surface in these two regimes have been defined in scaling and mean-field approaches. In the scaling scheme, the extension of the mushroom away from the surface is of the order of the Flory radius:

$$L_{mush} = R_F \cong aN^{3/5} \tag{1}$$

where N is the degree of polymerization (N = 46 for PEG-2000) and a is the monomer size (a = 3.5 Å for PEG-2000). According Equation 1, L_{mush} for PEG-2000 is about 35 Å.

The extension of the brush away from the surface depends on the concentration of the polymer. In the scaling scheme it is given by:

$$L_{sc} \cong N\frac{a^{5/3}}{D^{2/3}} \tag{2}$$

* In polymer theories the term "monomer" is used for the repeat unit in the polymerized chain. Materials science terminology distinguishes "monomer" as the original chemical species that is polymerized to form a polymer made of up to N repeating "mer" units.

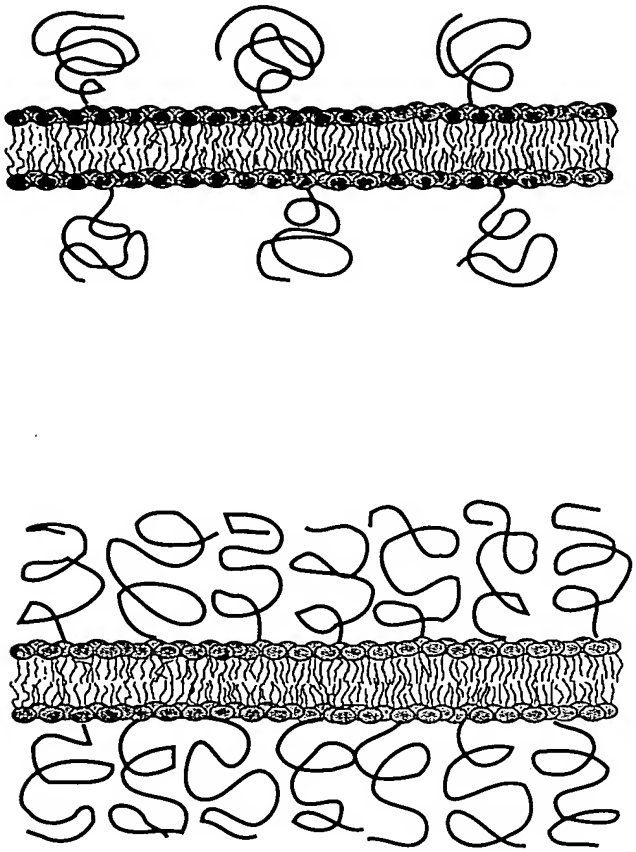

FIGURE 1. (a) Schematic diagram showing a polymer-grafted bilayer at low grafting concentration (mushrooms). (b) Schematic diagram showing a polymer-grafted bilayer at high grafting concentration (brush).

where D, the distance between points of grafting, is a measure of the polymer surface coverage.

In the mean-field scheme proposed by Milner et al., extension is given by:

$$L_{MWC} = \left(\frac{12}{\pi^2}\right)^{1/3} N \frac{a^{5/3}}{D^{2/3}} \tag{3}$$

A. MUSHROOMS

In the X-ray diffraction experiment two mushroom layers oppose each other. To introduce the scaling concepts for compression let us discuss the behavior of a single mushroom, compressed between two parallel solid plates. The question was first considered by de Gennes[23] and Daoud and de Gennes.[44] For strong compression (i.e., when the distance between the plates h is small compared to the random coil size of the polymer) they obtain for the free energy of the compressed polymer:

$$F_{conf}(h) \cong kTN\left(\frac{a}{h}\right)^{5/3} \tag{4}$$

The corresponding pressure (force per unit area) is

$$P_{conf}(h) = -\frac{1}{D^2}\frac{dF_{conf}}{d(h)} \cong \frac{kTN}{D^2 a}\left(\frac{a}{h}\right)^{8/3} \tag{5}$$

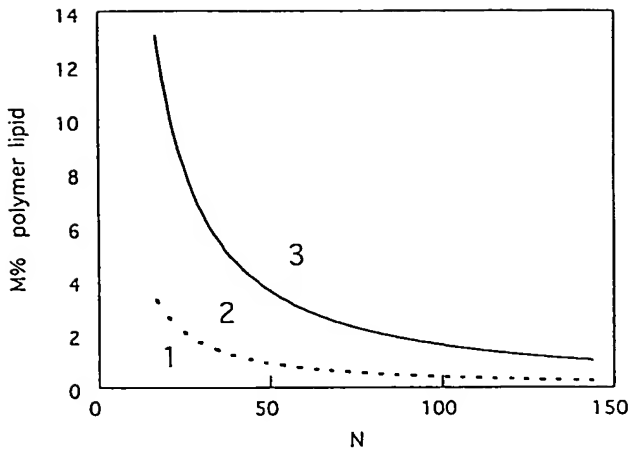

FIGURE 2. Regimes of polymer behavior: interdigitated mushroom, mushroom, and brush. (1) $D > 2R_F$ — interdigitated mushrooms; (2) $2R_F > D > R_F$ — mushrooms; (3) $D < R_F$ — brushes.

This formula describes the behavior of a strongly compressed mushroom. However, to interpret the full range of X-ray diffraction data from a fraction of an atmosphere applied pressure to pressure of several atmospheres it is important to know the polymer confinement energy when the distance between plates is close to the random coil size (i.e., small compressions). We suggest that in this case Flory's mean-field arguments are more appropriate.

Flory proposed that the energy of a random polymer coil in solution (for athermal solvent) has the form:

$$F = kT\frac{R_F^2}{Na^2} + kTa^3c^2R_F^3 \tag{6}$$

where $c = N/R_F^3$ is the monomer concentration inside the coil. The first term describes the entropy of the chain, the second accounts for the interactions between the monomers.

We assume that although the chain end is attached to the surface, the free energy of the polymer is given by Equation 6. The free energy has its minimum for $R_F = aN^{3/5}$, the Flory radius. If we compress the mushroom by a small amount with respect to its equilibrium size, it will respond with pressure which scales as:

$$P_{mf}(h) \cong \frac{kTN^{1/5}}{D^2}\left(\frac{R_F^3}{(h)^4} - \frac{h}{R_F^2}\right) \tag{7}$$

Now consider the experimental case of two compressed mushroom-covered surfaces in close proximity. For this case, depending on the distance D between points of grafting we define two subregimes of polymer behavior (see Figure 2):

1. $2R_F > D > R_F$ — mushrooms at low and high compression (region 2).
2. $D > 2R_F$ — interdigitated mushrooms at low and high compression (region 1).

We now consider the functional form of the steric pressure in each of these subregimes and under a range of compressive loads.

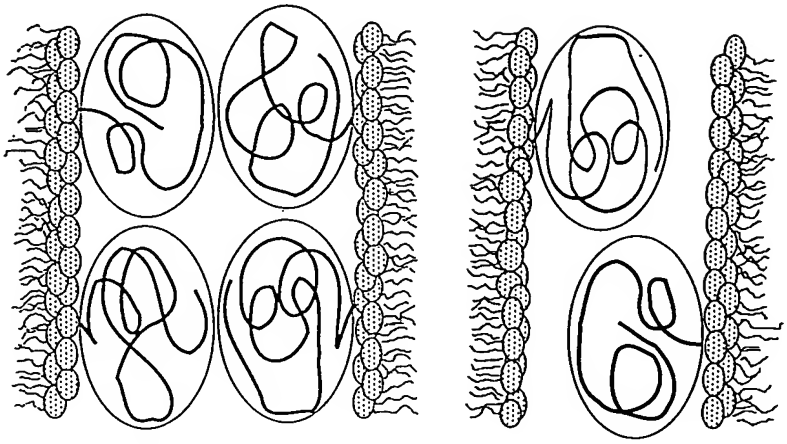

FIGURE 3. (a) Schematic diagram showing compressed mushrooms; (b) schematic diagram showing compressed interdigitated mushrooms.

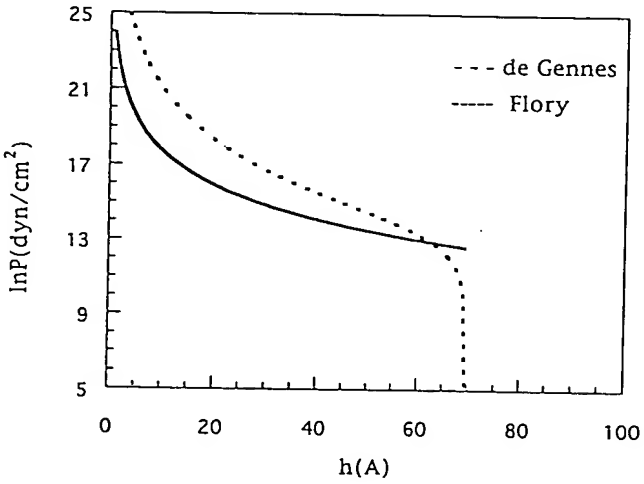

FIGURE 4. Theoretical pressure vs. interbilayer distance curves for polymer mushrooms: 1.2 *M%* polymer lipid, N = 46, *a* = 3.5 Å.

1. Mushrooms, Low Compression

If $2R_F > D > R_F$ the mushrooms on the opposite surfaces are dense enough so that they are squeezed one against the other (Figure 3a). We model this as compressing of each mushroom between two plates at separation h/2, where h is the separation between the bilayer surfaces — Equation 7 for $P_{mf}(h/2)$ holds. It is plotted in Figure 4 for N = 46, *a* = 3.5 Å, 1.2 *M%* polymer lipid for the whole range of compression.

2. Mushrooms, High Compression

The elastic energy of the highly compressed mushroom is described by P_{conf} (h/2), where P_{conf} is given by de Gennes' relationship (Equation 5) and is presented in Figure 4 for the whole range of compression for N = 46, *a* = 3.5 Å, 1.2 *M%* polymer lipid.

If we confine just one chain between two surfaces under the assumption that the local average monomer density does not change, the elastic energy is the only energy that opposes compression. A result of this compression will be the spreading of the mushroom (i.e., its size in the lateral direction $R_\parallel$ will increase). de Gennes computes this size to depend on the degree of compression as

$$R_{\parallel} \cong aN^{3/4}\left(\frac{a}{h/2}\right)^{1/4} \tag{8}$$

If the mushrooms are grafted on a surface at a given concentration, then at a certain h_{cr}, for which

$$R_{\parallel} \cong aN^{3/4}\left(\frac{a}{h/2}\right)^{1/4} = D \tag{9}$$

the squeezed mushrooms begin to overlap. Then, to compress the system further, we also need to overcome the osmotic repulsion in the surface polymer solution, which can now be considered semidilute. From Equation 9 we find h_{cr} to scale as:

$$\frac{h_{cr}}{2} = \frac{a^5N^3}{D^4} \tag{10}$$

The osmotic force per unit area for $h < h_{cr}$ is[44]

$$P_{osm}(h/2) = \frac{kT}{(h/2)^2 D} N^3\left(\frac{a}{D}\right)^5 \tag{11}$$

and the overall repulsion P_{sc} due to the compressed mushrooms for $h < h_{cr}$ will therefore be:

$$P_{sc} = P_{conf}(h/2) + P_{osm}(h/2) \tag{12}$$

where P_{conf} and P_{osm} are given by Equations 5 and 11, respectively.

3. Interdigitated Mushrooms, Low Compression

If $D > 2R_F$ the bilayers are sparsely covered by polymer. When two surfaces approach it would appear that a mushroom grafted to one of them can be compressed directly against the other surface; i.e., the mushrooms from opposite bilayers can interdigitate (Figure 3b). In the case of 100% interdigitation, each mushroom is compressed between two plates of separation h. If we assume that the degree of interdigitation depends on the surface coverage, two mushrooms will interdigitate with probability $(D - R_F)/D$ (note that D is greater than $2R_F$). Then the average force will be:

$$P(h) = \frac{D - R_F}{D} P_{mf}(h) + \frac{R_F}{D} P_{mf}(h/2) \tag{13}$$

Note that the force between mushrooms is steeper than interdigitated mushrooms. The major assumption here is that the mushrooms do not move on the surface. This is true if lipid diffusion in the plane of the bilayer does not occur. In the gel-phase lipid system the diffusion is slow, but still present. The diffusion coefficient is on the order of 10^{-10} cm^2/s. Let us calculate the average distance that a lipid moves in the course of t = 12 h, which is a characteristic time for the equilibration of the multilamellar liposomal system used in the X-ray diffraction equipment. This distance is given by the formula:

$$\bar{x} = \sqrt{2Dt} \tag{14}$$

where D is the diffusion coefficient. We find $\bar{x}$ to be of the order of 30 μm so in the course of these 12 h lipids will mix well. Since there is compression applied, this compression may

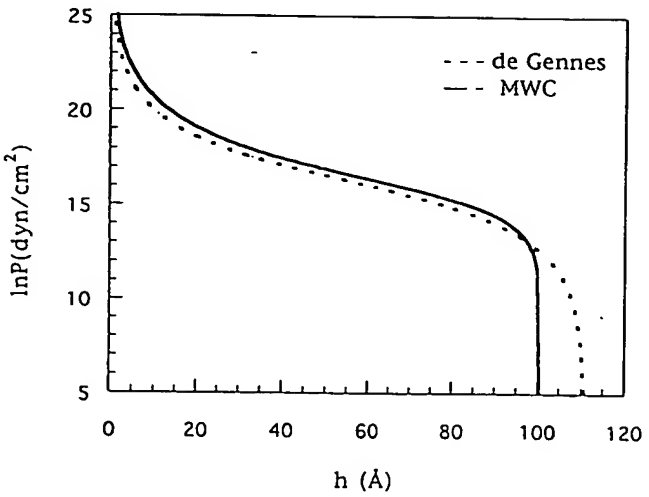

FIGURE 5. Theoretical pressure vs. interbilayer distance curves for polymer brushes: 12 *M%* polymer lipid, N = 46, *a* = 3.5 Å.

lead to lipid rearrangement such that a 100% interdigitation is achieved. For low compression, however, this effect might not be significant. In the case of a 100% interdigitation the average force is given by $P_{mf}(h)$ (Equation 7).

4. Interdigitated Mushrooms, High Compression

In this case, we assume that due to high compression polymer-lipids rearrange such that a 100% interdigitation occurs. The repulsive force will be given by Equation 5.

B. BRUSHES

de Gennes[28] and Alexander[29] consider the physical response of the polymer when two brush-covered surfaces are brought in close contact. The repulsive pressure is found to depend on the distance between the two surfaces h as:

$$p_{sc}(h) \cong \frac{kT}{D^3}\left[\left(\frac{2L_{sc}}{h}\right)^{9/4} - \left(\frac{h}{2L_{sc}}\right)^{3/4}\right] \tag{15}$$

Milner and coworkers[31-34] calculate the repulsive force in terms of their mean-field theory to be:

$$P_{MWC}(h) = \frac{5}{9D^2} F_{MWC}\left(\frac{L_{MWC}}{(h/2)^2} - \frac{h}{L_{MWC}^2} + \frac{(h/2)^4}{L_{MWC}^5}\right) \tag{16}$$

where F_{MWC} is given by:

$$F_{MWC} = \frac{9}{10} kT\left(\frac{\pi^2}{12}\right)^{1/3} N \frac{a^{4/3}}{D^{4/3}} \tag{17}$$

The two theoretical predictions are plotted in Figure 5 for N = 46, *a* = 3.5 Å, 12 *M%* polymer lipid.

These theoretical schemes are now being tested. The predictions are being compared to X-ray data. The comparison between theory and experiment is to be published elsewhere.

IV. PHASE BEHAVIOR OF THE POLYMER-LIPID/LIPID MIXTURE IN AQUEOUS MEDIUM AND MAXIMUM CONCENTRATION OF POLYMER-LIPIDS IN THE BILAYER

As suggested by the presented theory and experimental studies,[9] increasing the concentration of grafted polymer, as well as the molecular weight of the polymer, further improves the repulsive properties of the lipid bilayer surfaces, creating a denser, larger brush. The brush, however, will eventually perturb the surface that is grafted to such that at high concentrations of polymer-lipid a transition must occur from a bilayer to a micellar phase.[9] We propose two possible scenarios for this transition: (1) the transition can be due to reaching critical material parameters of the bilayer, or (2) it can be determined by the thermodynamics of the self-assembling amphiphilic system. For each scenario we define a critical concentration: (1) n_{sat} (which we will call the saturation limit of polymer-lipid in the bilayer) is determined by the material characteristics of the bilayer, and (2) n_{tr} (which will be called the thermodynamic crossover) is the polymer-lipid concentration for which micelle formation becomes energetically favorable.

We will now discuss the two possible schemes for micelle formation. First, we note that since by definition there are no lateral interactions between polymers in the mushroom regime the phase transition to micelles which is determined by polymer lateral interactions should not occur until polymer concentrations are within the brush regime.

A. PHASE TRANSITION, DETERMINED BY THE MATERIAL PROPERTIES OF THE BILAYER

In the brush regime there is energy stored in the brush which increases with the molecular weight of the polymer and the grafting concentration. This stored free energy is expressed as a lateral tension between the brushes. If the bilayer structure is stable, the lateral pressure in the two polymer layers (see Figures 1b and 6) will be felt as an isotropic tension in the bilayer. Just as in micropipette experiments[15,42] where applied tension leads to area change, the polymer will cause area expansion of the bilayer, which will be opposed by the cohesive forces in the bilayer (Figure 6). This tension will increase with the molecular weight and the grafting concentration and eventually reach the critical tension that the bilayer can support as a material. The maximum concentration will therefore be the one at which the critical material parameters are attained; concentrations higher than this one cannot be achieved. So the material properties define a saturation limit n_{sat}. Increasing the polymer-lipid concentration above n_{sat} will lead to a transition from bilayer into a mixed bilayer and micellar phase. The excess polymer-lipids are expected to segregate to form micelles. These micelles have been observed in an electron micrograph for a 100% polymer-lipid.[9]

To calculate the maximal concentration of polymer-lipid in the bilayer we developed a simple lateral force model.[45] We suppose that the maximum concentration of polymer-lipid in the bilayer is the one at which the lateral steric repulsion between the polymer chains equals the bilayer tensile strength τ_s, measured by micropipette experiments.[15] The prediction is plotted in Figure 7 as a function of cholesterol content in the SOPC bilayer.

Based on this prediction we expect then that we can control and manipulate this maximum concentration by changing both the polymer molecular weight and the lipid composition (e.g., incorporating cholesterol that is known to increase the tensile strength τ_s of the bilayer[15] or using gel-phase lipids). Thus, we propose that increasing the bilayer cohesion is a unique means of achieving a high density of grafting in these self-assembling lipid systems.

The saturation concentration due to these material properties gives the absolute limit of polymer concentration in the bilayer. If the lipid molecules formed only bilayers then this would be the only scheme for the saturation limit. In fact, lipid molecules are polymorphic. (Lipid polymorphism is the ability of the lipid molecules to form different types of aggregates under different conditions.) As pointed out by Israelachvili,[46] fully hydrated lipid molecules

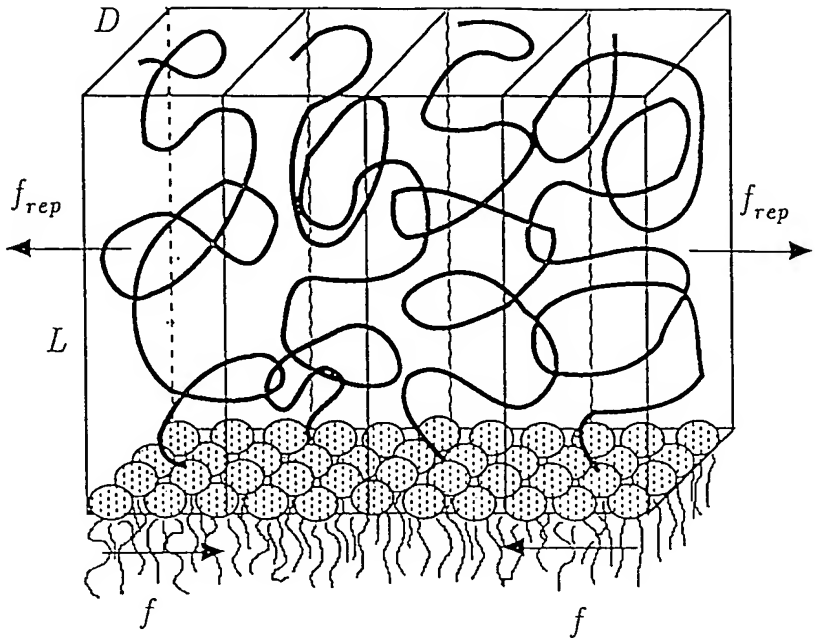

FIGURE 6. Schematic diagram showing that the brush lateral pressure is balanced by bilayer cohesion.

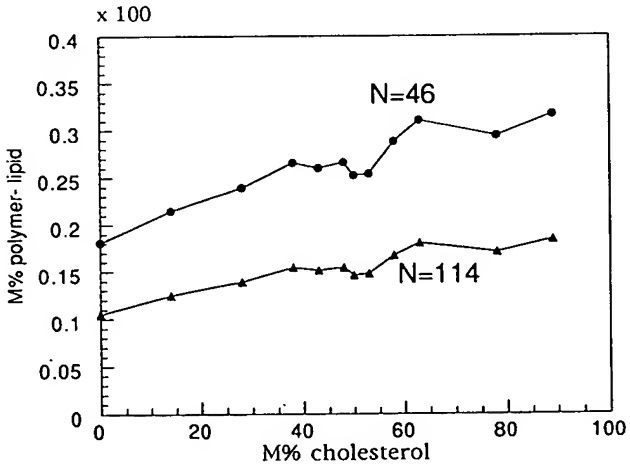

FIGURE 7. Maximum concentration of polymer-lipid that can be incorporated in the SOPC bilayer as a function of cholesterol content.

such as DSPC, egg PC, etc., which have a predominantly cylindrical shape (the area per head equals the area per tail) will form bilayers. But changes in the temperature, pH, or, as discussed here, high concentration of incorporated polymer-lipid which leads to enhanced repulsions between the lipid headgroup, can induce a phase transition to more stable micellar or cubic phases.

B. PHASE TRANSITION DETERMINED BY THE THERMODYNAMICS OF A SELF-ASSEMBLING POLYMER/LIPID/LIPID SYSTEM (THE MINIMUM ENERGY REQUIREMENT)

Curving the grafting surface will relax the lateral tension in the polymer layer. The higher the curvature, the bigger the relaxation. The highest curvature will be obtained if the lipids

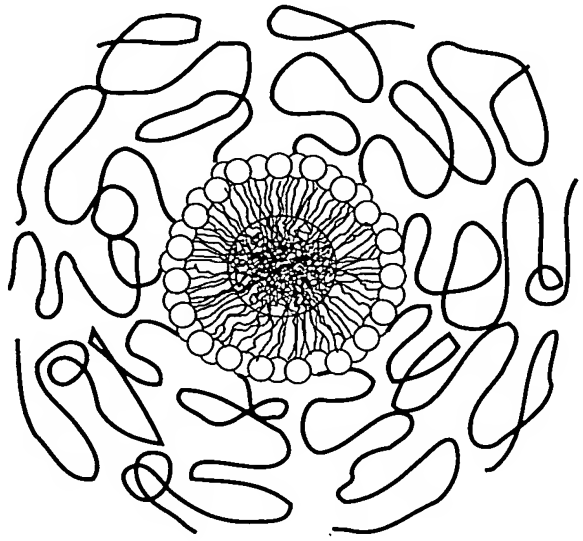

FIGURE 8. Schematic diagram showing a polymer-grafted micelle.

pack into micelles (cylindrical or spherical) (Figure 8). This will cost additional energy due to "unfavorable packing" of the hydrocarbon chains. While the latter energy does not depend on the molecular weight and grafting density, the energy stored in the polymer brush will depend crucially on them: the bigger the molecular weight of the polymer, the bigger the relaxation when a polymer-lipid is transferred from a bilayer to a micelle. That is why, for high molecular weight, the brush energy decrease due to curving of the surface will be bigger than the hydrocarbon energy increase due to micelle packing. A phase transition from bilayers to micelles will therefore occur. The concentration n_{tr} (the thermodynamic crossover) at which this occurs will depend on the molecular weight and the grafting density of the polymer and on the structure of the hydrocarbon tails: for instance, number of CH_2 groups and number of double bonds, which determines how "difficult" it is to pack the chains in a micelle.

To understand the mechanism of this phase transition we study the changes in the energy of the system that occur upon an increase in the concentration of the polymer-lipids in the lipid mixture. We obtain that, upon an increase in the concentration of polymer-lipid in the sample, the energy minimum gradually moves from a bilayer into a micellar phase.

For concentrations lower than n_{tr} the dominant phase is the bilayer. Increasing the concentration above the thermodynamic crossover n_{tr} has the effect of decreasing the probability for formation of bilayers. Above n_{tr} a mixed phase exists — bilayers and micelles have the same energy and coexist. So the transition from bilayers to micelles is not a first-order transition.

Polymer-lipid concentration in the bilayer is equal to the system concentration (i.e., concentration in the original anhydrous mixture) for concentrations less than n_{tr}. Above n_{tr} it is equal to n_{tr}, as dictated by the behavior of a mixed-phase region.

In summary, in the first scheme, the phase transition from bilayers to micelles is determined by the critical parameters of the bilayer as a material. In the second one, the minimum energy requirement is the driving force for the phase transition. Both schemes predict micelle formation and the existence of a mixed phase above a certain critical polymer-lipid concentration. Also, in both schemes the polymer-lipid concentration in the bilayer increases up to this critical concentration and then levels off. In the first scheme this concentration is the saturation limit of the polymer-lipids in the bilayer n_{sat} and is determined by the material parameters of the bilayer. In the second scheme the critical concentration is the thermodynamic crossover n_{tr}.

Both schemes are possible. The one that occurs first (i.e., has a lower critical concentration) will determine the phase behavior of the system. This will depend on the material parameters and micelle-forming properties of the lipids that make up the bilayer. For phospholipids such as DSPC, SOPC, and their mixtures with cholesterol we obtain theoretically that n_{tr} is much lower than n_{sat}. (The results will not be presented here in detail, they are obtained numerically and are to be published elsewhere.) For molecular weight 2000 Da in gel-phase bilayers the concentration n_{tr} is about 15 *M%* and for liquid bilayers it is about 8 *M%*. Thus, the proposed analysis predicts that the driving force for the phase transition is the minimum energy requirement.

V. DISCUSSION

The theoretical analyses presented here and elsewhere[45] can provide valuable information that is directly related to the Stealth liposomes and their expected performance in service:

1. The concentration of polymer-lipid in the liposomes is not necessarily equal to the concentration in the anhydrous mixture. Bilayer concentrations are only equal to the concentration in the anhydrous mixture for concentrations less than n_{sat} or n_{tr}, whichever is less. Bilayers in the two phase region (bilayers and micelles) cannot have higher concentrations than these limits.
2. Increasing the polymer-lipid concentration above n_{tr} will induce micelle formation. Thus, in Stealth liposome preparations there is no point increasing the polymer-lipid concentration above n_{sat} or n_{tr}, whichever is less. (In our theoretical study we obtain that for molecular weights 2000 and 5000 Da $n_{sat} > n_{tr}$.) For molecular weight 2000 Da for gel-phase bilayers the concentration n_{tr} is about 15 *M%* and for liquid bilayers it is about 8 *M%*. Increasing the polymer-lipid concentrations above these values will not improve the repulsive properties of the bilayers and hence will not affect their circulation times.
3. The theoretical predictions show that the grafted polymer layer changes the elastic constants and the tensile strength of the bilayer.[45] These changes occur within the brush regime and make the bilayer weaker. It may appear that for the sake of stability it is better if the polymer chains barely touch and interact: i.e., at the very borderline between mushrooms and brushes, which is about 5% in liquid bilayers for PEG-2000.
4. The nature of the lipids in the bilayer appears to be of importance. The higher the cohesion, the more stable the system. This suggests that the mixture of saturated (gel-phase) lipids such as DSPC with cholesterol may be the optimum material for Stealth liposomes preparation.
5. For concentrations less than n_{sat} or n_{tr} the theory allows us to predict the extension of the polymer from the surface. It is given by Equation 1 for the mushroom regime and by Equation 3 for the brush regime. At these distances from the lipid surface the polymer steric interactions are "switched on" and their magnitude grows almost exponentially towards the surface, dominating over the electrostatic and van der Waals forces. For distances larger than L this steric interaction is zero. If $L \leq \lambda_D$, the Debye length (a condition achievable in low salt) there will be a long electrostatic tail to the repulsion.[47] If $L \gg \lambda_D$ the electrostatic contribution will be negligible. Then, if L is within the range of the van der Waals forces (we can expect this for molecular weights of 2000 and smaller at low surface coverage) some weak attraction between the polymer-covered bilayers may exist.[48] The extension L should also give an indication of the maximum size of PEG that can be incorporated in a targetable liposome such that a protein molecule attached to the surface can still be accessible for binding to its target molecule.

6. When considering the mechanism of repulsion between two identical polymer-covered bilayers, we showed that the polymer acts as a good repulsive barrier against the close approach of another surface. As some preliminary experiments suggest, however, the polymer layer is not an effective barrier against small proteins and surfactant molecules.[49] It appears that these small molecules can easily diffuse through the polymer layer. Thus, the liposome in circulation may well get opsonized, but the steric barrier that the polymer creates does not allow for the close approach of macrophages and other cells with phagocytic activity to the opsonized liposome surface. Thus, its long circulation time in the bloodstream is still maintained.

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